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Research article

Effects of exposure to volatile organic compounds (VOCs) content from paint on automobile paint workers in Nsukka, South Eastern Nigeria

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ABSTRACT

Background: Volatile organic compounds (VOCs) fume in the workplace can act as an inducing agent to many health disorders.
Objectives: This work investigated the effects of exposure to VOCs content from paint on the automobile paint workers in South Eastern Nigeria.
Methods: A total of fifty (50) respondent participated in the study. Following the completion of informed consent form and well-structured questionnaire, blood samples were drawn and used for biochemical analysis.
Results: The results of the haematological analysis showed a significant ($p < 0.05$) increase in white blood cell (WBC) cluster of differentiation 4 (CD4), and platelet (PLT), and a significant ($p < 0.05$) decrease in packed cell volume (PCV), hemoglobin (HB), lymphocytes (LYM) and eosinophil (EOS) of the exposed automobile paint workers compared to the control (unexposed workers). Results also showed significant ($p < 0.05$) increase in liver marker indices; alanine transaminase (ALT), aspartate transaminase (AST), total bilirubin (TB) and albumin (ALB) as well as significant ($p < 0.05$) decrease in Alkaline phosphatase (ALP), total protein (TP), direct bilirubin (DB). There was significant ($p < 0.05$) increase in urea, creatinine, potassium (K^{+}), uric acid and nitric oxide concentrations and decrease in sodium (Na^{+}) and bicarbonate (HCO^{3-}) Again, results showed significant increase in Glutathione (GSH), and Glutathione peroxidase (GPx) and significant ($p < 0.05$) decrease in Superoxide dismutase (SOD) and Catalase (CAT). The Malondialdehyde MDA concentration showed varied significant ($p < 0.05$) difference based on ages. There was significant ($p < 0.05$) increase in luteinizing hormone (LH) and Follicle stimulating hormone (FSH), and significant ($p < 0.05$) decrease in the Testosterone (TET) concentrations of the exposed automobile paint workers compared to the unexposed workers.
Conclusions: Result of this study suggests a toxic outcome due to exposure to VOCs in spray paint workers.

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1. Introduction

Occupational exposure to volatile organic compounds (VOCs) content in the work place is one of the leading causes of health disorder among exposed workers [1]. Automobile workshops in Nsukka South Eastern Nigeria are populated largely by male artisans that carry out their day-to-day activities through labour intensive method. They work long hours under very poor working environment, lack of personal protective equipment (PPM), and poor automobile spray rooms. These paints are composed of different kinds of VOCs that project danger to human health [2].

The workers are exposed to a myriad of these fumes during paint spray through inhalation and sometimes through ingestion and dermal contact. These chemical substances from paint induces generation of free radicals that can trigger oxidative stress [3].Unregulated generation reactive oxygen spices in the body as a result of occupational exposure is the hub of many health disorder such as liver damage, renal disorder, lipid peroxidation, cell mutation, immunological imbalance and cell apoptosis [4]. The magnitude of the effect of the exposure to VOCs is dependent on the nature of chemical substance, duration and method of exposure [5].

Automobile spray is a mixture of paint and thinner added for easy evaporation and drying when applied. They release myriads of VOCs such as acetone, xylene, ethylbenzene, styrene, methylethylketone and toluene depending on the chemical components of the spray paint. The poorly ventilated workshop can enhance painters' exposure to variety of VOCs contained in paint during paint spray, enabling inhalation of 50 % of the emitted VOCs during automobile spray.[6]Therefore, this study investigated possible toxic effect of exposure to VOCs from paint on exposed automobile workers in Nsukka South Eastern Nigeria using biochemical, immunological, haematological and hormonal indices.

2. Materials and methods

2.1. Study design

A cross-sectional study employing quantitative, specimen analysis design was undertaken among randomly selected automobile paint spray workers at the mechanic village, Nsukka Enugu State Nigeria.

2.2. Area of study

The study was conducted at the mechanic village in Nsukka. Nsukka is a town in Enugu State, South-East Nigeria. It lies between latitude 6°, 18′ and 70.00, North and longitude 6°, 52 and 70, 541 east. Nsukka town hosts the prestigious University of Nigeria, the first indigenous Nigerian university.

2.3. Selection of the study population

The proposed study targeted automobile paint workers exposed to volatile organic compounds from paint spray at the mechanic village Nsukka.

2.4. Inclusion criteria

Apparently healthy automobile paint workers exposed to volatile organic compounds from paint spray for more than one month period and are male at the mechanic village Nsukka, who consented to participate in the study were included in the study.

2.5. Exclusion criteria

i. All automobile paint workers exposed to volatile organic compounds from paint spray but are not males
ii. All automobile paint workers exposed to volatile organic compounds from paint spray who are apparently sick or suffer from an underlying sickness
iii. All automobile paint workers exposed to volatile organic compounds from paint spray who refuse to consent to participate in the study

2.6. Sample size determination

Following the Kish Leslie formula:

$N = Z^2P(1\text{-}P)/E^2$, where n = estimated minimum sample size required and P = proportion of a characteristic in a sample (50 %). Z = 1.96 (for 95 % confidence interval), e = the margin of error set at 5 %.

$N = 1.96^2x0.5(1–0.5)/0.05^2$ N = 25

2.7. Sampling technique

The study employed the purposive sampling technique until the required sample was reached. The technique gave each member of the target population an equal and independent chance of being selected for the study. This ensured that the selected sample was a good representative of the whole population.

2.8. Data collection methods

A structured interviewer-administered questionnaire was used in the English language; however, it was translated into the local language (Igbo) for those who did not understand English. Data was collected with the help of trained research assistants from the study area who were trained first on how to collect data before the process began. The questionnaires were filled out by the respondents in the study. Blood samples were collected by trained medical personnel.

2.9. Ethical considerations

The study was carried out after approval by the Chairperson of Ethics Committee on the recommendation of the Faculty of Biological Sciences Research Ethics Committee (FBSREC), University of Nigeria, Nsukka (UNN), with the following certificate reference number: UNN/FBS/23/PhD/2017/246868. The approval was explained to the persons in charge of the mechanic village granted permission to conduct the study in their area of jurisdiction. All the information gathered was kept confidential and anonymous. The purpose of the study was explained, and informed consent was secured from each worker who participated. Workers involvement in this study was voluntary, and participants who were not willing to participate in the study and those who wished to withdraw from the study at any stage were allowed to do so.

2.10. Data analysis

Data obtained from the study were analysed using IBM Statistical Product and Service Solution (IBM-SPSS) version 21 (Chicago, IL). Significant differences in the means were established by one-way analysis of variance (ANOVA), Post hoc multiple comparisons, and Duncan's homogenous subset. The results were expressed as means $\pm$ standard deviation of replicate measurements. Mean values with $p \leq 0.05$ were considered significant.

2.11. Subject

A total of 50 samples from willing subjects were used (25 from automobile paint worker and 25 served as the control group). The exposed workers were categorized based on age and level of exposure. The control was unexposed automobile workers. All the individuals examined in this study participated in a face-to-face questionnaire which included standard demographic data (age, gender) as well as questions relating to medical issues (exposure to X-rays, vaccinations, medications), life style (smoking, coffee, alcohol, diet) and their occupation (number of hours worked per day, time/duration exposed to organic solvents, use of protective measures).

2.12. Blood collection

Five (5) ml of blood was collected by trained medical personnel and dispensed into appropriate tubes for both biochemical and hematological assays. Clotted blood samples on dispensed into plain tubes were centrifuged with aid of a centrifuge machine at 4000 rpm for 5 min and the resulted serum used for further analysis.

2.13. Biochemical analysis

2.13.1. Full blood counts analysis

The total blood count was determined with the aid of Automated Blood Cell Counter also known as Hematology Analyzer Machine (DxH 800) a product of Beckman Coulter as described by Ochei and Kolhatkar [6]. Briefly stated, well mixed whole blood sample was diluted with diluent reagent buffer and loaded into the machine via aspiration. After detection by flow cytometry, cells were identified, counted, analysed and reported in real time.

2.13.2. Liver function parameters

The impact of VOCs on activities some liver enzymes; ALT, ALP and AST in serum were determined using the methods of Huang et al., Reitman and Frankel, Englehardt [7–9]respectively. Other liver functions markers such as total protein, total bilirubin and albumin were determined using the method of Vatzidis, Gustafsson [10,11]respectively.

2.13.3. Procedure for determination of AST and ALT

Test tubes were labelled blank, standard, control and test. 0.5 ml of the substrate was introduced into all the test tubes. 0.1 ml of serum sample was added to the labelled test tubes, gently mixed and allowed to incubate for 30 min for ALT (and 60 min for AST) at 37 °C. To both tubes, 0.5 ml of the colour reagent was added, mixed and allowed to stand for 20 min. Then, 0.5 ml of NaOH was added

after 5 min and the absorbance read against blank at 540 nm.

2.13.4. Procedure for determination of ALP

Three test tubes were labelled as follows; blank, standard, control (test). 0.5 ml of ALP substrate was transferred into all the test tubes and incubated for 3 min at 37 °C. 0.1 ml of deionized water was used as blank and 0.1 ml of the blood serum and standard was added to other test tubes appropriately and incubated for 10 min at 37 °C. then 2.5 ml of colour reagent was added to the test tubes and were mixed. The absorbance of the mixture was read at 590 nm against reagent blank.

2.13.5. Procedure for determination of total serum protein concentration

Three test tubes were used to carry out this test; blank, standard and sample were labelled and to the sample tubes were added 0.02 ml of serum, to the standard test tube, a volume, 0.02 ml of protein standard was added and 0.02 ml water to the blank test tube. One milliliter of the protein reagent was added to the test tubes each. This was mixed well and left to stand for 25 min at room temperature (20–25 °C). The absorbance was taken at 540 nm.

2.14. Renal function indices

The effects of the exposure on urea, creatinine, K^+, Na^+ and HCO_3^- concentrations were determined using the methods of Taylor and Howard, Burtis, Tietz et al., [12–14]respectively

2.14.1. Procedure for determination of urea

A total of three test tubes were used for this assay, labelled as black, standard and sample. Prepared urease reagent (1 ml) was pipetted into the three test tubes then followed by 10 μL of distilled water into the blank, 10 μL of standard into standard test tube and 10 μL of plasma into plasma test tube. Each content of the test tubes was gently mixed and allowed to stand for 10 min at 37 °C, afterwards, absorbance reading was taken at a wave length of 540 nm against blank.

2.14.2. Procedure for determination of creatinine

This was analysed by deproteinizing 1 ml of sample plasma and 1 ml of creatinine standard in different test tuble with 1.0 ml of trichloroacetic acid respectively. The test tubes were centrifuged at 4000 rpm for 10 min and the resultant supernatant reacted with picric acid in an alkaline medium to form creatinine picrate. The reaction was allowed to stand for 2 min and then read against reagent blank at 492 nm.

2.14.3. Procedure for determination of bicarbonate ion ($HCO3^-$)

Bicarbonate reagent was prepared and 1 ml of it pipetted into three different test tubes labelled as blank, standard and sample. 10 μL of distilled water was added to the blank, 10 μL of bicarbonate standard to the test tube labelled standard and 10 μL of the plasma into the sample test tube. The three-test tube was gently missed and allowed to stand for 10 min at 37 °C and read at a wave length of 340 nm against water blank.

2.14.4. Procedure for determination of sodium ion (Na^+)

Filtrate reagent (1 ml) was pipetted into three well labelled test tubes; blank, standard and sample. 50 μL of standard was added into standard test tubes, 50 μL of plasma into sample test tube and 50 μL of distilled water into blank. The mixtures inside the three test tubes were shaking vigorously for 3 min, centrifuged for 10 min at a speed of 1500×*g*. The resultant supernatant was transferred into fresh labelled test tubes (blank, standard and sample) and 0.1 ml acid reagent added to each of the test tubes containing the supernatant respectively for colour development. The mixture was read at 550 nm against water blank.Sodium ion was then calculated using the formula below;

$$Sodium\ Conc\left(\frac{mEq}{L}\right) = \frac{Abs\ of\ Blank - Abs\ of\ Sample}{Abs\ of\ Blank - Abs\ of\ Standard} \times Standard\ Conc\ \left(\frac{150mEq}{L}\right)$$

2.14.5. Procedure for determination of potassium K^+

Potassium reagent 1 ml was pipetted into three test tubes labelled blank, standard and sample. 10 μL of potassium standard was added into standard test tube, 10 μL of plasma into sample test tubes and 10 μL of distilled water into blank respectively. The three setup was then gently mixed and allowed to stand for 3 min at 37 °C and read at 500 nm against reagent blank. Potassium conc was calculated using the formula below;

$$\text{Potassium Conc } (mEq/L) = \frac{Abs\ of\ Sample}{\text{Abs of Standard}} \times Standard\ Conc(4mEq/L)$$

2.15. Evaluation of oxidative stress markers

Oxidative stress parameters such as; GPx, SOD, CAT, GSH and MDA were determined by the method of Wallins et al., Bayfield and Cole, Aebi, Arthur and Boyne [15–18]respectively.

2.15.1. Procedure for determination of SOD activity

The supernatant (500 μl) was added to 0.800 ml of carbonate buffer (100 mM, PH 10.2) and 100ul of epinephrine (3 Mm). The change in absorbance of each sample was then recorded at 480 nm in spectrophotometer for 2 min at an interval of 15 s. Parallel blank and standard were run for determination SOD activity.

2.15.2. Procedure for determination of catalase

Catalase activity was determined by adding 0.1 ml of supernatant to cuvette containing 1.9 ml of 50 mM phosphate buffer (PH 7.0). Reaction was started by the addition of 1.0 ml of freshly prepared 30 Mm H_2O_2. The rate of decomposition of H_2O_2 was measured spectrophotometrically from changes in absorbance at 240 nm.

2.15.3. Procedure for estimation of reduced glutathione (GSH)

A volume 0.2 ml of sample was mixed with 1.8 ml of EDTA solution. To this 3.0 ml of precipitating reagent was added, mixed thoroughly and kept for 5 min before centrifugation. To 2 ml of the filtrate, 4 ml of 0.3 M disodium hydrogen phosphate solution and 1 ml of DTNB reagent were added and the colour developed was read at 412 nm in spectrophotometer. A set of standard solutions containing 20–100 μg of reduced glutathione was treated similarly. The values were expressed as mg/dL for plasma.

2.15.4. Procedure for determination of malondialdehyde (MDA) concentration

To 0.2 ml of test sample,0.2 ml of SDS, 1.5 ml of acetic acid and 1.5 ml of TBA were added. The mixture was made up to 4 ml with water and then heated in a water both at 95 °C for 60 min After cooling, 1 ml of water and 5 ml of n-butanol/pyridine mixture were added and shaken vigorously. After centrifugation at 4000 rpm for 10 min, the organic layer was taken and its absorbance was read at 532 nm. The level of lipid peroxides was expressed as moles of MDA released/g wet tissue.

2.16. Evaluation of reproductive hormones

Serum testosterone (TET) was determined according to the method of Chen [19] as contained in Callbioteckinc ELISA kit USA. Serum follicle stimulating hormone (FSH), luteinizing hormone (LH) and progesterone (PRO) and oestradiol (E2) were determined following methods described by Rose, Morimoto and Inouye, Qui et al. [20–22], respectively.

3. Results

(see Tables 1–6).

4. Discussion

Daily, humans are exposed to various kinds of pollutants with potential toxic health effects. The kind and extent of health effect from these pollutants are largely dependent on, concentration, nature of pollutants and route of exposure and exposure time. Occupational exposure could be the underlying cause or a triggering factor to one of the many health disorders among industry workers. GCMS result of the automobile paints used by automobile workers showed the presence of Xylene (21.98 %), toluene (17.35 %), ethanol (34.61 %) and different isomers of benzenes (48.01 %) at different concentration peaks. These compounds are believed to account for the adverse effects observed in the biochemical analysis of haematological indices of automobile painters. VOC's have been established to be hazardous compounds sometimes described as cancerous [23]. Result of haematological indices of automobile paint spray workers in Nsukka mechanic village showed significant ($p < 0.05$) increase in WBC and platelet count and significant ($p < 0.05$) decrease in PCV and HB across the different age groups compared to the control (unexposed automobile workers). The increase in the WBC (leukocytosis) could be as a result of the fight back mechanism against xenobiotics or inflammation from inhaled toxicants. Leukocytosis is naturally designed to serve as body immune response indicator for struggling immune system against pathogenicity. This is also supported by the significant increase in neutrophils count observed from the exposed group compared to unexposed group. The neutrophil is a major component of the white blood cell that helps in resolving infections and healing damage tissues.

Table 1
Effects of VOCs Content from Paint on Hematological parameters of Automobile Paint Workers.

Age group	PCV%	WBC $x10^{12}$/L	RBC $x10^{12}$/L	HB g/dL	Platelet $x10^{12}$/L
Exposed 18–25	44.67 ± 3.70^{b}	8.91 ± 0.65^{b}	5.50 ± 0.36^{a}	11.72 ± 4.03^{a}	237.78 ± 33.82^{b}
Unexposed	45.33 ± 3.71^{a}	6.38 ± 4.41^{a}	5.50 ± 0.36^{a}	17.20 ± 2.15^{ab}	220.55 ± 18.10^{ab}
Exposed 26–35	40.60 ± 1.95^{ab}	8.16 ± 0.96^{ab}	5.48 ± 0.16^{a}	10.32 ± 3.67^{a}	120.00 ± 30.82^{a}
Unexposed	48.40 ± 0.89^{a}	6.48 ± 1.46^{a}	5.48 ± 0.16^{a}	14.7 ± 3.50^{a}	202.00 ± 19.23^{a}
Exposed 36–45	37.13 ± 6.47^{a}	7.37 ± 1.23^{a}	5.28 ± 0.99^{a}	10.65 ± 3.08^{a}	110.00 ± 39.27^{a}
Unexposed	48.00 ± 1.51^{a}	6.25 ± 0.78^{a}	5.28 ± 0.99^{a}	15.62 ± 2.58^{ab}	229.37 ± 15.22^{b}
Exposed 46–55	39.33 ± 3.06^{ab}	7.06 ± 0.70^{a}	5.46 ± 0.25^{a}	9.90 ± 2.15^{a}	100.00 ± 36.05^{a}
Unexposed	48.67 ± 1.15^{a}	6.06 ± 0.70^{a}	5.46 ± 0.25^{a}	18.70 ± 1.13^{b}	216.65 ± 11.55^{ab}

Results are expressed in mean ± SD (n = 50). Mean values with different superscripts (a,b,c and d) across the column are considered significant at $p < 0.05$.

Table 2
Effects of VOCs content from paint on immunological indices of automobile paint workers.

Age group	CD_4%	Neutrophils%	Lymphocytes%	Eosinophils%	Monocytes%	Basophils%
Exposed18–25	668.86±263.17[a]	61.00±5.80[a]	37.86±6.64[a]	1.14±1.57[a]	0.00±0.00[a]	0.00±0.00[a]
Unexposed	575.57±240.75[a]	575.57±240.75[a]	62.86±3.02[a]	1.43±1.51[a]	0.00±0.00[a]	0.00±0.00[a]
Exposed 26–35	452.40±188.61[a]	64.89±3.35[a]	34.00±3.74[a]	1.20±1.10[a]	0.00±0.00[a]	0.00±0.00[a]
Unexposed	453.80±121.49[a]	453.80±121.49[a]	63.60±5.73[a]	1.60±1.67[a]	0.00±0.00[a]	0.00±0.00[a]
Exposed 36–45	570.17±256.85[a]	64.00±5.66[a]	33.33±6.25[a]	1.67±1.51[a]	1.00±1.10[a]	0.00±0.00[a]
Unexposed	600.00±208.05[a]	600.00±208.05[a]	66.00±4.38[a]	1.33±1.03[a]	0.00±0.00[a]	0.00±0.00[a]
Exposed 46–55	609.00±469.52[a]	66.00±5.66[a]	34.00±5.66[a]	0.00±0.00[a]	0.00±0.00[b]	0.00±0.00[a]
Unexposed	589.50±41.72[a]	589.50±41.72[a]	69.00±1.41[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]

Results are expressed in mean ± SD (n = 50), Mean values with different superscripts (a,b,c and d) across the column are considered significant at $p < 0.05$.

Table 3
Effects of VOCs content from paint on the liver marker indices of automobile workers.

Age group	ALT iu/L	ALP iu/L	AST iu/L	TP g/dL	DBil mg/dL	TBil mg/dL	ALB g/dL
Exposed18–25	17.00±8.10[a]	29.57±9.09[a]	21.71±9.96[a]	5.38±0.83[a]	0.23±0.08[ab]	0.81±0.41[a]	3.14±0.37[a]
Unexposed	11.43±11.29[b]	30.71±3.15[a]	8.43±2.51[b]	5.71±1.27[a]	0.24±0.04[a]	0.79±0.14[a]	3.11±0.54[a]
Exposed 26–35	17.00±10.84[a]	29.60±9.56[a]	19.60±10.09[a]	5.00±0.73[a]	0.33±0.13[b]	0.97±0.55[a]	3.60±0.56[a]
Unexposed	7.80±3.70[b]	31.80±3.96[ab]	8.00±4.95[b]	5.00±0.98[a]	0.24±0.09[a]	0.68±0.08[a]	3.16±0.34[a]
Exposed 36–45	10.50±5.24[a]	26.17±12.5[a]	23.33±10.27[a]	5.20±0.61[a]	0.27±0.09[ab]	1.04±0.53[a]	3.52±0.255[a]
Unexposed	7.50±3.08[b]	39.50±7.12[b]	8.17±2.99[b]	5.57±0.41[a]	0.27±0.06[a]	0.73±0.15[a]	3.48±0.34[a]
Exposed 46–55	24.00±15.56[a]	23.00±0.00[a]	21.50±14.85[a]	5.25±0.78[a]	0.15±0.01[a]	0.66±0.19[a]	3.35±0.21[a]
Unexposed	5.50±2.12[b]	32.50±6.36[ab]	5.00±1.41[b]	5.45±1.06[a]	0.25±0.13[a]	0.73±0.09[a]	3.20±0.71[a]

Results are expressed in mean ± SD (n = 50). Mean values with different superscripts (a,b,c and d) across the column are considered significant at $p < 0.05$.

Table 4
Effects of VOCs Content from Paint on Oxidative stress parameters of Automobile Paint workers.

Age group	MDA mg/mL	SOD U/mg	CAT U/mg	GSH mg/dL	GPx U/mg
Exposed 18–25	0.68±0.42[a]	11.43±0.04[a]	3.08±1.74[a]	3.64±1.99[a]	24.48±3.31[a]
Unexposed	0.55±0.24[a]	11.50±0.02[a]	8.02±5.06[a]	2.58±1.04[a]	16.66±4.14[a]
Exposed 26–35	0.66±0.43[a]	11.37±0.21[a]	1.88±1.48[a]	3.57±1.30[a]	21.81±5.29[a]
Unexposed	0.63±0.15[a]	11.47±0.04[a]	6.37±2.47[a]	2.49±1.20[a]	17.13±5.43[a]
Exposed 36–45	0.67±0.58[a]	11.36±0.22[a]	1.49±1.81[a]	3.39±1.12[a]	24.44±9.09[a]
Unexposed	0.34±0.20[a]	11.49±0.02[a]	7.92±3.89[a]	2.69±1.93[a]	16.66±2.31[a]
Exposed 46–55	0.72±1.00[a]	11.41±0.08[a]	1.26±1.61[a]	4.04±1.59[a]	30.23±22.56[a]
Unexposed	0.61±0.63[a]	11.47±0.042[a]	6.41±5.08[a]	2.67±1.47[a]	14.25±1.04[a]

Results are expressed in mean ± SD (n = 50). Mean values with different superscripts (a,b,c and d) across the column are considered significant at $p < 0.05$.

Hematotoxicity could be indication of exposure to pollutants as seen from the haematological results of the exposed automobile paint workers [24]. The low PCV count observed from the exposed group compared to automobile non-paint workers could indicate workers predisposition to anaemia due to toxic exposure. This is also supported by the low Hb count compared to that of the unexposed group. There was a significant increase in the CD4 count of the exposed paint workers compared to the automobile non paint workers and a significant decrease in lymphocytes and eosinophils of the exposed group compared to unexposed automobile workers. The CD4 is well established and known play a central role in immune protection [25] Therefore, the abnormal increase in the CD4 of the exposed group compared to the unexposed could indicate instability of the immune system. Poorly ventilated automobile workshop can cause hypoxia inhibiting tissue metabolism due to increased generation of carbon monoxide (CO) that binds to haemoglobin. Carbon monoxide has high binding affinity to oxygen in the haemoglobins subunit [26,27].Total WBC, and the differentials lymphocytes and neutrophils act as biomarkers to endothelial attack. The high WBC count from the exposed automobile paint workers could be a predisposition factor to atherosclerosis and cardiovascular diseases [28]The observed high platelet count could predispose to abnormal clots in blood vessels of exposed automobile painter workers. Also, high carbon monoxide in the body from paint fumes can induce platelets to produce more nitric oxide (NO) that reacts with superoxide and other reactive oxygen species to produce peroxynitrite that can lead to neurological diseases when it is dys-regulated [29].

The abnormality in the liver health markers of the exposed individuals compared to that of unexposed persons (control) could be sign of liver toxicity due to exposure to paint fumes [30]. The liver is the hub of metabolism of xenobiotics. The changes in the enzyme

Table 5
Effects of VOCs Content from Paint on some fertility hormones of Automobile Paint Workers.

Age group	Testosterone ng/mL	Luteinizing hormone miu/mL	Follicle stimulating hormone miu/mL
Exposed 18–25	5.07±1.65[a]	7.42±1.29[a]	7.42±1.29[a]
Unexposed	3.93±0.43[a]	9.65±2.57[a]	9.65±2.57[a]
Exposed 26–35	5.27±1.96[a]	7.26±1.52[a]	7.26±1.52[a]
Unexposed	3.69±0.48[a]	9.45±1.47[a]	9.45±1.47[a]
Exposed 36–45	4.87±2.36[a]	6.04±1.50[a]	6.04±1.50[a]
Unexposed	3.93±0.41[a]	7.43±1.28[a]	7.43±1.28[a]
Exposed 46–55	4.40±2.26[a]	5.87±1.68[a]	5.87±1.68[a]
Unexposed	3.81±0.65[a]	7.97±0.59[a]	7.97±0.59[a]

Results are expressed in mean ± SD (n = 50). Mean values with different superscripts (a,b,c and d) across the column are considered significant at $p < 0.05$.

Table 6
Effects of VOCs Content from Paint on Renal parameters of Automobile Paint workers.

Age group	Urea mg/dL	Creatinine mg/dL	K+ mmol/L	Na + mmol/L	Cl^-mmol/L	HCO_3^-mmol/L	Uric acidmmol/L	Nitric oxideug/dL
Exp 18–25	32.57±7.72[a]	1.61±0.24[a]	4.12	145.00	95.57	22.88±0.38	6.11±1.64[a]	7.53±14.33[a]
Unexposed	31.14 ±10.19[ab]	1.13±0.35[ab]	3.99 ±0.48[a]	158.43 ±14.19b	95.29 ±0.49a	25.50±0.36	5.60±0.80a	0.69±0.22[a]
Exp 26–35	35.80±5.40a	1.62±0.31[a]	4.44	142.60±7.77[b]	95.60	23.55±0.34	5.52±1.33[a]	1.74±0.58[a]
Unexposed	33.00±7.94[b]	1.18±0.35[b]	±0.49[a] 3.90 ±0.44[a]	152.60 ±16.21[b]	±1.14[a] 95.60 ±0.55[a]	27.30±0,88	5.36±0.84[a]	0.73±0.20[a]
Exp 36–45	39.33±3.93[a]	1.65±0.06[a]	3.98	137.00	96.33	23.87±0.43	5.68±1.27[a]	1.50±0.74[a]
Unexposed	30.17 ±9.85[ab]	1.07±0.34[ab]	±0.67[a] 3.98 ±0.57a	±20.87[ab] 134.66±17.24 ab	±1.37[a] 95.50 ±0.55a	26.46 ±0.35	5.55±0.60a	0.69±0.16[a]
Exp 46–55	40.00±4.41b	1.65±0.07[a]	4.60	109.00±1.41[a]	96.00	24.90±0.67	6.10±2.69[a]	1.45±0.32[a]
Unexposed	17.50±4.95[a]	0.60±0.14[a]	±0.85[a] 3.75 ±0.35[a]	125.00±18.38[a]	±1.41[a] 97.50 ±0.71[b]	28.44±0.77	4.70±1.13[a]	0.64±0.02[a]

Results are expressed as mean ± SD (n = 50). Mean values with different superscripts (a,b,c and d) across the column are considered significant at $p < 0.05$.

activities from our study could mean a demonstration of hepatotoxicity caused by inhaled VOCs suspended in automobile paint fumes [31]. Human exposure to VOCs such as formaldehyde used in paint formulations can induce hepatotoxicity [23], toluene in relatively high concentration has been shown to inhibit the transport of bile acid in the liver [30]. There was a significant increase in ALT and AST activities of the exposed paint workers compared to the control group. This abnormal increase especially in ALT level could indicate liver toxicity. ALT enzymes are mainly found in the liver unlike other liver functions enzymes such as ALP and AST. It is worthy to note the important role of ALT in bioconversion of amino acid to ketoacids, therefore abnormal increase in ALT level may affect amino acid metabolism in the liver [32] This finding is in agreement with the work of Iruka et al. [33] Non-significant increase in total bilirubin and albumin concentrations and decrease in serum total protein concentration of the exposed paint workers compared to non-paint automobile workers, are equally indicative of liver toxicity and in line with an earlier report by Somayeh et al. [34].

Significant increase in Creatinine, potassium ion (K^+), bicarbonate ion (HCO_3^-), nitric oxide (NO), uric acid and a significant decrease in sodium ion (Na^+) concentration in the blood of the automobile paint workers compared to the non-paint workers observed in the study appeared to increase from younger to older age groups. The significant decrease in plasma sodium ion level could indicate a disruption in electrolyte balance which might affect its role in nerve and muscle regulations [35]. Again low sodium level could be a risk factor for kidney failure and cognitive impairment [35].

The increase in urea and creatinine, a by-product of protein metabolism could indicate impairment of the renal system function. The high serum uric acid concentration (hyperuricemia) from our study, could suggest susceptibility to arthritis due to clumping of uric acid in joints. This might be responsible for common complaint of joint pains by automobile paint workers as shown by some responses in the questionnaire. This could also be indicative of kidney damage owing to inability of the kidneys to filter dissolved uric acid in the blood as urine. The high nitric oxide may be due to constant exposure to paint fumes and their ability to combine with VOCs [36]. This could be the reason for symptoms such as headache, fatigue, dizziness after a prolong period of unprotected exposure to paint fumes.

Male reproductive hormones such as testosterone (TET), FSH and LH were analysed. Serum TET concentration was significantly ($p < 0.05$) increased in automobile paint workers compared to the non-paint workers. The observed decrease in LH and FSH hormones may indicate sexual dysfunction and in long run led to infertility [37]. LH is essential in the regulation of TET synthesis in the body

while FSH modulates spermiocytogenesis and spermiogenesis in male organisms [38]. However, the increase in TET may indicate increase in sexual urge or demand since it is responsible for maintenance of secondary sexual traits and development of male sex organs [37]. Scientific evidence has shown that human exposure to paint fumes can inhibit the activity of gonadotropin, responsible for the release of LH and FSH hormones from the pituitary glands [1].

The report of our study showed an increase in oxidative stress parameters such as MDA, GSH and GPx and a reduction in SOD and CAT of exposed automobile paint workers compared to non-automobile paint workers. Scientific studies have shown that human exposure to paint fumes can activate generation of reactive oxygen species (ROS) [38]. The increase in MDA which is a precursor to lipid peroxidation could indicate tissue damage. This is as a result of increase in peroxidation and breakdown of the human antioxidant defence mechanisms [38]. The increase in GSH and GPx could be a compensatory mechanism due to toxicity, in order to mop high level of reactive oxygen species (ROS). They help in detoxifying the body system from lipid peroxides and H_2O_2 by reducing H_2O_2 to H_2O and O_2 [39]. The effect of paint on exposed automobile paint workers on the tested biochemical parameters increased with ascending order of ages. This could be attributed to aging factors. Age is associated with decline in metabolic activity, reducing the body's ability in handling toxicity such as detoxification and elimination of toxin from the body. Again increased body fat content seen in elderly people could affect the distribution of fat-soluble toxins, often leading to prolonged or accumulative effect due to increased exposure time. The effect could also, be traced to decrease in enzyme activity and low immune system associated with ageing.

4.1. Conclusion

This study has revealed significant propensity for toxicity to automobile paint spray workers due to undue exposure to paint fumes. This poses serious health concern and calls for urgent action on the part of the workers, policy makers and the general public. Attitudinal change and adherence to safety rules are seriously advocated among these workers.

Data availability

Data have not been previously deposited on any public repository. All the data generated in this study have been included in the manuscript.

Ethics declaration

This study was reviewed and approved by the Chairman of Ethics Committee, on the recommendation of Faculty of Biological Sciences Research Ethics Committee (FBSREC), University of Nigeria, Nsukka (UNN) with certificate reference number: UNN/FBS/23/PhD/2017/246868 and dated February 16, 2023.

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CRediT authorship contribution statement

John Onyebuchi Ogbodo: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Simeon Ikechukwu Egba:** Writing – review & editing, Writing – original draft, Investigation, Data curation, Conceptualization. **Chizaramekpere Grace Ogbodo:** Formal analysis. **Ikechukwu Emmanuel Onwurah:** Writing – review & editing, Supervision, Project administration. **Obioma Uzoma Njoku:** Writing – review & editing, Supervision, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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